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Inflammation and microalbuminuria in nondiabetic and type 2 diabetic subjects: The Insulin Resistance Atherosclerosis Study

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Background. Microalbuminuria is a risk factor for cardiovascular disease, but the underlying pathomechanisms are still poorly understood. A relationship between C-reactive protein (CRP), a sensitive marker of inflammation, and atherosclerotic disease has been reported recently.

Methods. We hypothesized that microalbuminuria might be associated with chronic inflammation and investigated the relationship of urinary albumin excretion, as assessed from the albumin-to-creatinine ratio (ACR), in an untimed morning urine specimen, and two inflammatory markers (CRP and fibrinogen) in the large, triethnic population of the Insulin Resistance Atherosclerosis Study (IRAS). After exclusion of subjects with macroalbuminuria, 1481 subjects were studied.

Results. Both inflammatory markers were related to urinary ACR ($r = 0.17$ for CRP and $r = 0.14$ for fibrinogen, both $P = 0.0001$), an association that remained significant after adjustment for demographic variables, diabetic status, smoking, and use of angiotensin-converting enzyme inhibitors ($P < 0.01$). Mean levels of CRP and fibrinogen were elevated in microalbuminuric ($N = 262$) versus normoalbuminuric ($N = 1219$) subjects (5.37 ± 0.47 vs. 3.80 ± 0.15 mg/L and 295.7 ± 4.0 vs. 278.2 ± 1.6 mg/dL, both $P < 0.0001$). The associations were consistent among nondiabetic and type 2 diabetic subjects and among the three ethnic groups of the IRAS (non-Hispanic whites, blacks, Hispanics). In a logistic regression model, fibrinogen was independently associated with microalbuminuria ($P = 0.047$), along with hypertension, female gender, waist circumference, and fasting blood glucose, while CRP was not independently related to microalbuminuria in this model ($P = 0.26$).

Conclusion. We have shown an association of CRP and fibrinogen with urinary albumin excretion in the microalbuminuric range in type 2 diabetic and nondiabetic individuals. Chronic inflammation therefore emerges as a potential mediator between microalbuminuria and macrovascular disease.

Key words: cardiovascular disease, albumin-to-creatinine ratio, C-reactive protein, urinary albumin excretion, nephropathy, end-stage renal disease.

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Microalbuminuria, defined as an increased urinary albumin excretion rate in the absence of clinically compromised renal function, is a strong risk factor for overt nephropathy in patients with type 1 diabetes [1, 2]. In contrast, in patients with type 2 diabetes, microalbuminuria predicts cardiovascular rather than renal disease [3–7]. In nondiabetic subjects, microalbuminuria was associated with excess early [8] and long-term [9–11] cardiovascular mortality. The reasons for the increased cardiovascular risk in subjects with microalbuminuria are largely unknown. It has been suggested that microalbuminuria generally reflects a state of widespread endothelial dysfunction and/or vascular damage [12].

Recently, a relationship between C-reactive protein (CRP), a sensitive marker of inflammation, and the development of atherosclerotic disease has been observed in experimental [13–15] and clinical studies [16–23]. While it is still unknown whether elevated CRP levels [and/or its main stimulator interleukin-6 (IL-6)] are the result of prevalent atherosclerotic disease or are actively involved in the initiation and/or progression of disease, the latter seems likely given the known functions of CRP [15, 24] and the recently identified role of IL-6 in mouse models [25].

In the present study, we hypothesized that microalbuminuria in type 2 diabetic and nondiabetic subjects might be related to markers of chronic inflammation, such as CRP. Previously, inflammatory markers were elevated in subjects with end-stage renal disease [23]; however, knowledge about a possible relationship of inflammation with albumin excretion in the non-nephropathic range is limited and controversial. In type 1 diabetic subjects with microalbuminuria and macroalbu-

minuria, no differences in CRP levels, compared with normoalbuminuric patients and healthy controls, were observed [26]. In type 2 diabetes, serum levels of sialic acid, another inflammatory marker, were elevated with increasing albumin excretion in one study [27], but were unrelated to microalbuminuria in another [28].

A potential mediator of urinary albumin excretion as well as cardiovascular disease is increased insulin resistance, which has been associated with microalbuminuria in diabetic subjects [29–31], their relatives [32], and nondiabetic subjects [33]. More recently, insulin resistance has also been related to inflammatory markers [34, 35].

In the present study, we investigated the relationship of microalbuminuria to inflammatory markers in a large triethnic population of type 2 diabetic and nondiabetic individuals. We included potential confounding factors that were associated with albumin excretion and/or inflammation in previous studies, including insulin resistance, as directly measured by a frequently sampled intravenous glucose tolerance test (FSIGT), blood pressure (BP), and measures of body fat mass (BMI) and body fat distribution (waist circumference).

METHODS

The Insulin Resistance Atherosclerosis Study (IRAS) is a multicenter epidemiologic study exploring relationships between insulin resistance, cardiovascular risk factors, and cardiovascular disease across different ethnic groups and varying states of glucose tolerance. A full description of the design and methods of the IRAS has been published previously [36]. The IRAS protocol was approved by local institutional review committees, and all subjects gave informed consent.

A total of 1625 individuals participated in the IRAS. Subjects with a current acute illness (including clinically significant infectious disease) were excluded from IRAS examination. Urinary albumin excretion in the normoalbuminuric and microalbuminuric range was the scope of the present report; therefore, subjects with macroalbuminuria [albumin-to-creatinine ratio (ACR) $\geq$ 20 mg/mmol] were excluded from the analyses. Thus, this report includes data on 1481 subjects, in whom the ACR as well as CRP and fibrinogen levels were assessed. Height and weight were measured following a standardized protocol. Body mass index [weight/height^2 (kg/m^2)] was used as an estimate of overall adiposity, and the minimum waist circumference was considered an estimate of visceral fat mass. Cigarette smoking was dichotomized into "never" and "ever" (including past and current) using a standard questionnaire. BP was measured according to a standard procedure, and hypertension was defined as systolic BP $\geq$140 mm Hg and/or diastolic BP $\geq$90 mm Hg or current use of antihypertensive medication.

The IRAS examination required two visits. Patients were asked prior to each visit to fast for 12 hours, to abstain from heavy exercise and alcohol for 24 hours, and to refrain from smoking the morning of the examination. A standard 75 g oral glucose tolerance test was performed, and the glucose tolerance status was based on the World Health Organization criteria [37].

Insulin sensitivity was assessed by a FSIGT [38] with minimal model analysis [39]. Two modifications of the original protocol were used. An injection of regular insulin, rather than tolbutamide, was used to ensure adequate plasma insulin levels for the accurate computation of insulin sensitivity across a broad range of glucose tolerance [40], including diabetic patients because of their blunted or absent insulin response. In addition, the reduced sampling protocol (which required 12 rather than 30 plasma samples and shows results similar to the full protocol) [41] was used because of the large number of subjects. Insulin sensitivity, expressed as the insulin sensitivity index (S_I), was calculated by mathematical modeling methods (MINMOD, version 3.0, 1994). This modified version of the FSIGT protocol used in the IRAS has been compared with the hyperinsulinemic euglycemic clamp and has been shown to be an adequate estimate of insulin resistance [42].

Laboratory measurements

Urinary albumin and creatinine concentrations were assessed in a random morning spot urine sample. Urinary albumin was measured from samples stored at -20°C by a commercial immunoprecipitation assay (Incstar SPQ test system, Stillwater, MN, USA) with a sensitivity of 5.8 mg/L and intra-assay and interassay coefficients of variation of 1.50 and 1.80%, respectively. Urinary creatinine was determined by a modified Jaffe method [43]. Urinary albumin and creatinine for all samples were measured at the central IRAS laboratory at the Medlantic Research Institute (Washington, D.C., USA). From 170 blind duplicate specimens, the external coefficient of variation was 12% for urinary albumin measurements and 17% for urinary creatinine measurements; the correlation between the two blind duplicate measurements was 0.82 for urinary albumin and 0.71 for urinary creatinine [33]. The urinary ACR (albumin in milligrams per liter and creatinine in millimoles per liter) was used as a measure of albumin excretion. Overnight ACR correlates well with urinary albumin excretion [44, 45], and ACR measured in a single untimed urine specimen has been shown to be an effective means for identifying diabetic patients who are at risk of developing overt nephropathy [46]. An overnight ACR $\geq$2 mg/mmol predicts an albumin excretion rate $>$30 μg/min with a high sensitivity and specificity [44]. Microalbuminuria was defined as ACR between 2 and 20 mg/mmol [33].

C-reactive protein was measured by in-house ultrasensitive competitive immunoassay (antibodies and anti-

gens from Calbiochem, La Jolla, CA, USA) with an interassay CV of 8.9% [47]. Fibrinogen was measured in citrated plasma with a modified clot-rate assay using the Diagnostica STAGO ST4 instrument, as described previously [48]. This was based on the original method of Clauss with a CV of 3.0% [49]. Samples for fibrinogen and CRP were prepared, frozen, and stored at −70°C at the clinical centers not later than 90 minutes after blood drawing. Frozen samples were shipped on a monthly basis to the Laboratory for Clinical Biochemistry Research, University of Vermont (Burlington, VT, USA), where measurements were performed. Glucose and insulin levels were measured as published previously [50].

Statistical analyses

Statistical analyses were performed using the SAS statistical software system. We analyzed urinary albumin excretion as a continuous (ACR) as well as a dichotomous (normoalbuminuria vs. microalbuminuria) variable. Spearman rank correlations were calculated between ACR and fibrinogen and CRP (Table 2). Stepwise partial rank correlations were performed adjusting for age, gender, ethnicity, clinic, and diabetic status (model B), smoking, use of angiotensin-converting enzyme (ACE) inhibitors (model C), BMI, waist (model D), hypertension (model E), and S_I (model F). These analyses were performed to determine the extent to which a relationship was altered after adjusting for potentially confounding covariates, previously shown to be related to albuminuria or inflammatory marker or both. In the IRAS, previously we have shown an association of S_I and measures of body fat and microalbuminuria [33], CRP [35], and fibrinogen [50], respectively. Glycemia and BP are important determinants of albuminuria [51], and smoking and use of ACE inhibitors [51] are further potential determinants. Smoking has also been related to CRP levels [18, 22].

Next, to compare mean values of CRP and fibrinogen in subjects with normoalbuminuria versus microalbuminuria, analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were performed (Table 3). For these analyses as well as the interaction models, logarithmically transformed values of CRP were used because the distribution of the residuals from the fitted models became normally distributed after log transformation. Unadjusted and adjusted means for log of CRP (shown back-transformed on Table 3) and fibrinogen were calculated using analogous stepwise adjustments as for the Spearman rank correlations. Finally, to determine the relative contribution of variables to urinary albumin excretion, a logistic regression analysis with microalbuminuria as the outcome variable was performed. Two analogous models for log of CRP and fibrinogen were fit. Independent variables were age, gender, ethnicity, clinic, diabetic status, smoking, use of ACE inhibitors, BMI, waist circumference, hypertension, fasting glucose, S_I, and finally log of CRP or fibrinogen (Table 4). All analyses were performed in the overall population and were also stratified by gender, ethnicity, and diabetic status. We also tested for possible interactions of gender, ethnicity, and diabetic status, respectively, on the association of urinary albumin excretion (ACR, microalbuminuria) with CRP and fibrinogen. We included in separate models interaction terms for gender × ACR, gender × microalbuminuria, ethnicity × ACR, etc. These models were adjusted for age, gender, ethnicity, clinic, and diabetic status. *P* values of less than 0.05 (two sided) were considered statistically significant.

Table 1. Characteristics of the normo- (MAB−), and microalbuminuric (MAB+) subjects in the Insulin Resistance Atherosclerosis Study (IRAS)

	MAB−	MAB+	*P* value
N	1219	262	
Diabetic/nondiabetic %	28/72	48/52	0.0001
Hypertensive/nonhypertensive %	35/65	52/48	0.0001
Smokers/nonsmokers %	44/56	41/59	NS
Women/men %	54/46	63/37	0.01
Age *years*	55.5 ± 0.2	55.9 ± 0.5	NS
BMI kg/m^2	29.1 ± 0.2	31.2 ± 0.4	0.0001
Waist *cm*	92.5 ± 0.4	97.6 ± 1.0	0.0001
SBP *mm Hg*	120.5 ± 0.5	128.7 ± 1.1	0.0001
DBP *mm Hg*	76.8 ± 0.2	79.4 ± 0.6	0.0001
Fasting glucose *mg/dL*	118.2 ± 1.2	141.9 ± 3.9	0.0001
$S_I \times 10^{-4}\ min^{-1} \cdot \mu U^{-1} \cdot mL^{-1}$	1.79 ± 0.06	1.16 ± 0.08	0.0001
Urinary albumin *mg/L*	8.38 ± 0.2	41.6 ± 2.9	0.0001
ACR *mg/mmol*	0.80 ± 0.01	4.93 ± 0.22	0.0001
CRP *mg/L*	3.80 ± 0.15	5.37 ± 0.47	0.0018
Fibrinogen *mg/dL*	278.2 ± 1.6	295.7 ± 4.0	0.0001

Values are means ± SE. Differences between MAB− and MAB+ were calculated by Student's *t*-test and chi-square test as appropriate.

Abbreviations are: *N*, number of study subjects; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; S_I, insulin sensitivity index; ACR, albumin-to-creatinine ratio; CRP, C-reactive protein.

RESULTS

Table 1 shows the descriptive data stratified by albuminuria status. Type 2 diabetes, hypertension, and female gender were more prevalent among subjects with microalbuminuria. Subjects with microalbuminuria had higher ACR, BMI, waist circumference, fasting glucose, CRP, and fibrinogen, and lower S_I.

Urinary albumin excretion as a continuous variable (ACR)

Both CRP and fibrinogen were significantly related to ACR in the overall population (Table 2). The association was somewhat weakened after adjusting for demographic variables and diabetic status (model B) and slightly further after adjustment for measures of body fat (model D), and hypertension (model E), but adjustment for smoking, use of ACE inhibitors, and S_I had no substantial effect

Table 2. Unadjusted (model A) and partial (models B–F) Spearman correlation coefficients of albumin-to-creatinine ratio (ACR) and inflammatory markers

Adjustment models	CRP	Fibrinogen
A: Unadjusted	0.17[a]	0.14[a]
B: Age, gender, ethnicity, clinic, diabetic status	0.09[b]	0.08[b]
C: + Smoking, ACE inhibitors	0.09[b]	0.08[b]
D: + BMI, waist	0.07[c]	0.07[c]
E: + Hypertension	0.06[c]	0.06[c]
F: + S_I	0.06[c]	0.06[c]

Overall population ($N = 1481$).

Covariates as shown were fit into the respective models in addition to all covariates included in the previous models in order to demonstrate to what extent the relation of inflammatory markers to albuminuria was attenuated by the respective covariates.

Abbreviations are: CRP, C-reactive protein; ACE, angiotensin-converting enzyme; BMI, body mass index; S_I, insulin sensitivity.

[a] $P = 0.0001$, [b] $P < 0.005$, [c] $P < 0.05$

Table 3. Mean values of inflammatory markers according to the albuminuria status (MAB+, microalbuminuria; MAB−, normoalbuminuria) in the overall population

Adjustment models	CRP *mg/L*			Fibrinogen *mg/dL*		
	MAB+	MAB−	*P* value	MAB+	MAB−	*P* value
A	2.94	2.01	**0.0001**	295.7	278.2	**0.0001**
B	2.80	2.23	**0.0012**	293.2	280.2	**0.0007**
C	2.80	2.20	**0.0010**	293.7	280.4	**0.0005**
D	2.39	2.12	0.08	287.3	278.4	**0.016**
E	2.36	2.18	0.20	287.0	279.4	**0.041**
F	2.29	2.10	0.24	286.5	278.9	**0.049**

Adjustment models include: A, unadjusted; B, age, gender, ethnicity, clinic, diabetic status; C, + smoking, ACE inhibitors; D, + BMI, waist; E, + hypertension; and F, + S_I.

Covariates as shown were fit into the respective models in addition to all covariates included in the previous models in order to demonstrate to what extent the relation of inflammatory markers to albuminuria was attenuated by the respective co-variates. Abbreviations are in Table 1.

on the association (models C and F). A similar pattern was also observed in the stratified analyses by gender, ethnicity, and diabetic status (data not shown).

Stratified analyses

By gender. The association of ACR with both inflammatory markers was significant in both men and in women. Although the correlation was slightly stronger in men than in women, no significant interaction of gender on the association of log CRP and fibrinogen with ACR and microalbuminuria, respectively, was found (interaction terms: $P = 0.4$ for both gender × microalbuminuria and gender × ACR, respectively, on log CRP, and $P = 0.7$ and $P = 0.14$ for gender × microalbuminuria and gender × ACR, respectively, on fibrinogen).

By ethnicity. The association of ACR with both inflammatory markers was significant in all three ethnic groups of the IRAS. These associations were slightly more pronounced in blacks than in non-Hispanic whites and Hispanics; however, no significant interaction of ethnicity on the association of log CRP and fibrinogen with ACR and microalbuminuria, respectively, was found (interaction terms: $P = 0.11$ and $P = 0.19$ for ethnicity × microalbuminuria and ethnicity × ACR, respectively, on log CRP, and $P = 0.6$ for both ethnicity × microalbuminuria and ethnicity × ACR, respectively, on fibrinogen).

By diabetic status. Correlation coefficients were comparable in diabetic and nondiabetic subjects. Interaction analyses showed no significant interaction of diabetes status on the association of CRP and fibrinogen, respectively, with ACR and microalbuminuria, respectively (data not shown).

Urinary albumin excretion as a dichotomous variable: Normoalbuminuria versus microalbuminuria

Inflammatory markers were higher in subjects with microalbuminuria versus normoalbuminuria (Tables 3 and 4). The differences were somewhat smaller after adjusting for demographic variables and diabetic status (Table 3, model B), measures of body fat (model D), and hypertension (model E). Further adjustment for S_I did not alter the results substantially (model F).

C-reactive protein and fibrinogen levels were also consistently higher in microalbuminuric subjects than in normoalbuminuric subjects when stratified by gender, ethnicity, and diabetic status (data not shown).

Logistic regression analysis (Table 4) showed that hypertension, fasting glucose, waist circumference, and gender were significant determinants of microalbuminuria (when log of CRP was considered as an independent variable in addition to age, gender, ethnicity, clinic, diabetic status, smoking, use of ACE inhibitors, BMI, waist circumference, hypertension, fasting glucose, and S_I), whereas gender, fasting glucose, hypertension, waist circumference, and fibrinogen were significantly related to microalbuminuria when fibrinogen was considered as an additional independent variable (Table 4).

DISCUSSION

We report an association of chronic inflammation with microalbuminuria in both nondiabetic and diabetic subjects in the IRAS. The association was significant in men and in women and across the three ethnic groups of the IRAS (blacks, non-Hispanic whites, Hispanics) and was similar in magnitude in these subgroups as judged by the nonsignificant tests of interaction. The association was moderately independent of confounding covariates such as body fat, hypertension, and S_I, although the strong covariance in these variables makes it difficult to estimate physiological independence statistically. Furthermore, another variable (either unmeasured and/or not included in the multivariate models) might account

Table 4. Logistic regression analysis with microalbuminuria as the dependent variable

Independent variable	Odds ratio	95% CI	*P* value
A. Log of CRP			
Hypertension (0, no; 1, yes)	1.82	1.33, 2.47	0.0002
Fasting glucose *10 mg/dL increment*	1.07	1.04, 1.08	0.0003
Waist circumference *5 cm increment*	1.09	1.03, 1.16	0.0058
Gender (0, male; 1, female)	1.57	1.46, 1.68	0.0067
Log CRP *10% change*	1.01	1.00, 1.02	0.26
B. Log of fibrinogen			
Fasting glucose *10 mg/dL increment*	1.07	1.03, 1.11	0.0001
Hypertension (0, no; 1, yes)	1.80	1.32, 2.45	0.0002
Waist circumference *5 cm increment*	1.08	1.02, 1.15	0.0070
Gender (0, male; 1, female)	1.52	1.11, 2.08	0.0067
Fibrinogen *10 mg/dL increment*	1.03	1.01, 1.04	0.047

After forcing age, gender, clinic, ethnicity, and diabetic status into the model, smoking status, use of ACE inhibitors, BMI, waist circumference, hypertension, fasting glucose, S_I, and log of CRP (A) or fibrinogen (B) were analyzed as independent variables. Only independent variables with a P value < 0.05 were left in the final fitted model and are shown on the table (except log CRP in model A). CI is confidence interval.

for the relationships as shown acting as a yet unidentified mediating factor.

Few previous studies have reported associations of inflammatory markers with urinary albumin excretion. Studies investigating levels of fibrinogen in populations of healthy [52, 53], hypertensive [54], type 1 [26, 55–57], and type 2 diabetic [57–60] populations have yielded conflicting results. Elevations of fibrinogen levels in microalbuminuric versus normoalbuminuric subjects were found in type 1 diabetes [56], Japanese type 2 diabetic subjects [60], nondiabetic hypertensive subjects [54], and a general population [52]. Other studies report elevated fibrinogen levels in subjects with albuminuria only in the macroalbuminuric range in patients with type 1 [26, 55, 57] and type 2 diabetes [57]. Finally, some studies found no differences in fibrinogen levels with respect to microalbuminuria in healthy subjects [53] and type 2 diabetes [58, 59]. These findings indicate that in patients with diabetes, levels of fibrinogen seem to be particularly high with progression of the disease (as indicated by macroalbuminuria [26, 55, 57] or retinopathy [55]). In nondiabetic populations, fibrinogen levels were significantly elevated in the presence of microalbuminuria in two studies [52, 54], and in one other, fibrinogen was also elevated but without reaching statistical significance ($P < 0.1$) [53]. These apparent discrepancies may be explained by differences in sample size, populations studied (nondiabetic vs. diabetic, severity of diabetes), and statistical adjustments performed in order to account for differences in potentially confounding covariates.

In the present study, we demonstrate elevated fibrinogen levels in subjects with microalbuminuria, even after adjusting for various potential confounding factors, previously shown to determine both microalbuminuria and fibrinogen levels, such as overall and central obesity, hypertension, and insulin resistance.

Previous studies about the relationship of microalbuminuria with more specific inflammatory markers other than fibrinogen are scarce and conflicting. In type 2 diabetes, microalbuminuria was associated with elevated levels of fibronectin [61] and sialic acid [27], whereas in another study, an association of microalbuminuria and sialic acid was only found in 26 Chinese, but not in 29 Indian and 24 Malaysian individuals [62], and in another small study ($N = 20$), no association of microalbuminuria with sialic acid was found at all [28]. CRP levels have only been studied in a small sample of type 1 diabetic patients showing no significant increase in the presence of microalbuminuria ($N = 22$) versus normoalbuminuria ($N = 17$) [26].

C-reactive protein, because of its biological properties (such as stable plasma levels over time) and the availability of highly sensitive assays, is a particularly useful marker of chronic, subclinical inflammation [63]. In the present study, we have shown that CRP levels were associated with low level albuminuria as analyzed as a continuous (ACR) as well as a dichotomous (microalbuminuria) variable. This association was attenuated with adjustment for body fat and hypertension, whereas S_I did not show a significant impact on these relationships. In a previous report from the IRAS, we have shown an independent association between S_I and microalbuminuria [33], in contrast to the multivariate regression models as shown on Table 4. In these latter models, however, we have analyzed (*1*) additional independent variables (smoking, use of ACE inhibitors, and, notably, the inflammatory markers); (*2*) a different measure of body fat (waist circumference vs. BMI, the former being stronger associated with S_I); and (*3*) different populations (different states of glucose tolerance vs. nondiabetic subjects exclusively).

We offer several possible explanations for our finding of an association of microalbuminuria with chronic inflammation. These explanations are not necessarily exclusive, and a considerable overlap presumably exists. Furthermore, we would like to emphasize that because

of the cross sectional character of the present study, we can only speculate about the causal and temporal relationships between chronic inflammation and albuminuria.

First, elevated levels of inflammatory markers may be the result of pre-existing atherosclerosis in subjects with microalbuminuria. In nondiabetic as well as type 2 diabetic subjects, microalbuminuria is associated with increased cardiovascular morbidity and mortality [3–11], suggesting that in individuals with microalbuminuria, atherosclerotic disease prevails. Second, elevations of acute phase proteins and/or inflammatory cytokines may directly alter glomerular function and thus be causally involved in the development of microalbuminuria. In previous studies, urinary albumin excretion has been elevated in inflammatory diseases [64–66] as well as in a variety of acute syndromes, such as trauma, burn injury, surgery, and acute myocardial infarction [8, 67–69]. Third, there is a potential link between inflammatory cytokines and glomerular function. Experimental data suggest that tumor necrosis factor-α induces glomerular infiltration by leukocytes [70] and that IL-1 and tumor necrosis factor-α influence the metabolism of glycosaminoglycans [71], which are components of the vascular endothelium and the glomerular basement membrane and are also involved in the etiology of microalbuminuria [72] and possibly macrovascular disease [73]. Finally, microalbuminuria and elevated levels of inflammatory proteins may have a common antecedent, namely the increased elaboration of inflammatory cytokines. Interestingly, in a recent report from the Hoorn study, microalbuminuria and pre-existing atherosclerotic disease independently predicted cardiovascular and all-cause mortality, suggesting that microalbuminuria or biological alterations associated with microalbuminuria affect mortality risk through a mechanism distinct from generalized atherosclerosis [74].

Finally, we would like to mention potential weaknesses of our study. First, the measurement of urinary albumin excretion in the IRAS is based on a single-spot urine sample. A prolonged sampling period (overnight or 24 h) or three measures instead of a single one, although difficult to perform in a large number of subjects, would have been preferable. However, a spot urine sample has been proposed as an accurate estimate of "true" albumin excretion [44–46], including in a black population [75], and is, therefore, in addition to the simple sampling procedure, particularly useful for epidemiological studies. Misclassification resulting from the sampling procedure is likely to weaken the relationship as shown, suggesting that the true relations may in fact be stronger. Furthermore, we believe that our results are strengthened by the fact that similar results were obtained when analyzing albumin excretion both as a continuous and as a dichotomous variable, thus minimizing potential misclassification bias (normoalbuminuria vs. microalbuminuria). Second, because of the relatively small number of subjects with microalbuminuria, we may have missed significant differences and relationships, especially in the subgroup analyses and in the multivariate statistical models.

Both CRP and microalbuminuria have been associated with cardiovascular disease in a number of previous studies. We have shown that chronic inflammation was associated with microalbuminuria in diabetic as well as nondiabetic subjects. We therefore propose chronic inflammation as a possible mediator between microalbuminuria and macrovascular disease. However, these data await confirmation in populations different from the IRAS, and a number of new and critical questions emerge. First, are levels of circulating inflammatory cytokines indeed elevated in relationship to microalbuminuria in nondiabetic and/or diabetic populations? Is the effect of currently available treatment shown to decrease urinary albumin excretion in microalbuminuric patients (such as antihypertensive drugs, particularly ACE inhibitors, improved glycemic control in patients with diabetes) [51] at least partly mediated by inflammation? This question seems of particular interest in light of the results of the Heart Outcomes Prevention Evaluation (HOPE) trial, showing a beneficial effect of an ACE inhibitor on the incidence of cardiovascular disease in a high-risk population [76]. Finally, in nondiabetic as well as diabetic microalbuminuric subjects, is there a beneficial effect of anti-inflammatory treatment on urinary albumin excretion and maybe even macrovascular disease? Further studies are clearly needed to address these issues.

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